Circadian Rhythms: An Electric Jolt to the Clock

The animal circadian pacemaker is composed of two transcriptional feedback loops, which regulate electrical activity in circadian neurons. Surprisingly, a new study reports that electrical activity can reprogram circadian transcription, and identifies CREB proteins as candidates for this reprograming.

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In animals, the molecular circadian pacemaker consists of two interlocked transcriptional feedback loops [1]. These two loops drive antiphasic waves of output gene transcription, which impact cell metabolism, physiology and behavior. For the pacemaker neurons that control circadian behaviors such as the sleep/wake cycle, membrane physiology and neuronal activity are critical circadian outputs [2–4]. Do these rhythmic outputs feedback on the molecular pacemaker to contribute to its oscillations? And do they modulate downstream clock-controlled genes? In this issue of *Current Biology*, Mizrak *et al.* [5] examine these important questions by looking at the consequences to the *Drosophila* circadian transcriptome of manipulating membrane properties of circadian neurons.

Ten years ago, Nitabach *et al.* surprisingly found that when they electrically silenced the *Drosophila* circadian pacemaker neurons (the small ventral lateral neurons; sLNvs), the molecular clock stopped functioning under constant conditions [6]. Hence, they proposed that proper electrical membrane physiology is needed for self-sustained molecular circadian rhythms, at least in circadian neurons. Interestingly, the resting membrane potential of the sLNvs shows a rhythm during the course of the day; it is hypopolarized near dawn and becomes hyperpolarized after dusk [2]. This raises the possibility that electrical activity rhythms could feed back on the transcriptional pacemaker to contribute to its oscillations and affect circadian output gene rhythms.

So what happens to the circadian pacemaker, and to the expression of the genes it controls, if one forces membrane activity to be constantly low (evening-like), or constantly high (morning-like)? This is the question Mizrak *et al.* [5] elegantly addressed. They expressed the potassium channel Kir to electrically silence the sLNvs, or the bacterial sodium NachBac channel to increase their neural activity [7] (Figure 1). The authors then took the technically challenging approach of isolating mRNAs from larval sLNvs (only eight neurons per brain!) at different time points, and performed whole genome expression studies to examine the consequences of manipulating electrical activity on the sLNv transcriptome. Two very interesting and provocative conclusions were drawn. First, there is a strong enrichment of genes under circadian control in the pool of genes whose expression was altered by membrane excitability manipulation. Second, there is a strong correlation between the directionality of the change in response to electrical manipulation and the time at which a gene is normally expressed. Indeed, the pattern of transcription in the morning becomes more evening-like when an evening-like neural activity is induced by Kir expression, while circadian transcription in the evening becomes more morning-like when morning-like neural activity is rendered through NachBac expression (Figure 1). Even the mRNA levels of circadian pacemaker genes were affected, although frequently not with the evening/morning logic just described. Thus, changes in electrical activity can somehow bypass, or at least modulate, the transcriptional program driven by the circadian pacemaker, and the authors interpret their results as an indication that electrical activity encodes time information.

These new data give support to previous studies promoting the idea that membrane physiology is an important part of the time-keeping mechanism in clock neurons [6,8]. However, a study published in *Current Biology* at the end of last year challenges the notion that electrical activity is required for circadian pacemaker function [9]. Depetris-Chauvin *et al.* [9] were able to turn Kir expression on and off in sLNvs, and showed that this results in temporary electrical silencing. As in the earlier study in which sLNvs were permanently silenced, flies became behaviorally arrhythmic, which is expected since the pacemaker neurons cannot fire action potentials. However, once Kir expression was blocked, circadian rhythms not only reemerged, but strikingly did so with the exact same phase they had before disappearing. Immunostaining revealed that oscillations of the key pacemaker protein PERIOD (PER) actually persisted during electrical silencing, as long as this silencing was not done for too long a period of time.

Thus, electrical activity does not appear to be required for circadian pacemaker function in clock neurons. Nevertheless, it is entirely possible that during reversible electrical silencing, many of the genes shown by Mizrak *et al.* [5] to be under electrical activity control are actually misregulated. Moreover, *per* (and *timeless*) mRNA levels are much less sensitive to membrane physiology manipulations than those of circadian output genes [5]. This would explain why PER protein cycling is almost unaffected by inducible electrical silencing. Electrical activity would thus encode time-of-day information, but this information would be primarily used to control output gene rhythms. It is, however, important to keep in mind that the manipulations made to electrical activity by Mizrak *et al.* are quite extreme, with prolonged hypo- and hyperexcitability that are probably well beyond the daily range of fluctuation in wild-type flies. The strikingly different results obtained between inducible and constitutive Kir expression are a warning that such manipulations are not without potential caveats [6,9]. Thus, in the future, it will be important to find ways to measure gene expression levels when membrane properties are more mildly altered. Another issue that would be interesting to address is the extent to which altered electrical activity *per se* contributes to the transcriptional changes observed in sLNvs. Indeed, these neurons form a network with other circadian neurons, exchanging

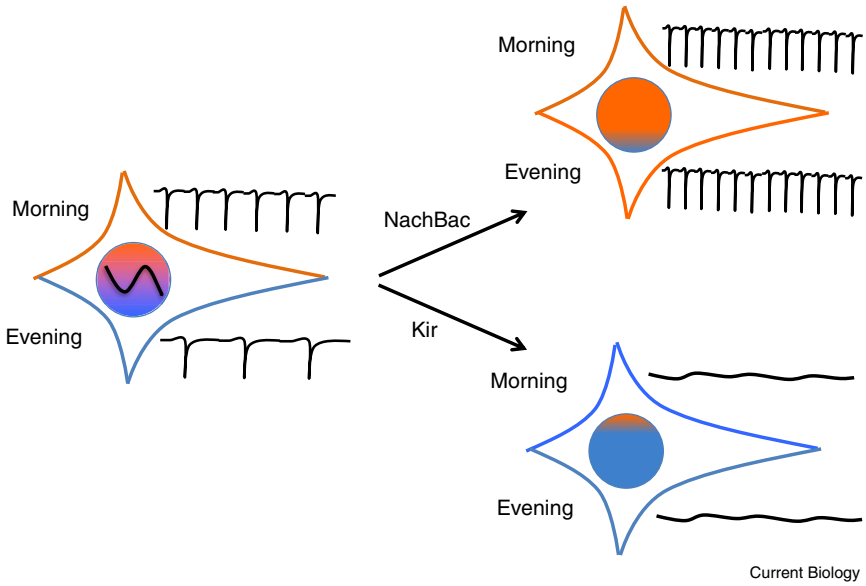

Figure 1. The circadian clock and electrical activity.

A wild-type circadian pacemaker neuron (sLNv) is represented on the left. Its membrane potential is depolarized in the morning, and hyperpolarized in the evening. Neuronal activity (represented by the frequency of spikes) is thus greater in the morning. This physiological rhythm and rhythms in gene expression are driven by the circadian clock (sine line). The orange color in the nucleus represents the 'morning' gene expression pattern, the blue color the 'evening' gene expression pattern. Expression of NachBac in sLNvs (upper right) increases neuronal activity and promotes morning gene expression in the evening (increased orange surface in the nucleus). On the contrary, Kir expression (lower right) hyperpolarizes the membrane and suppresses electrical activity, and promotes evening gene expression in the morning (increased blue surface in the nucleus).

signals with them [10,11]. Changes in sLNv activity should thus alter the inputs they receive from other circadian neurons, and this could also impact circadian transcription in the sLNvs.

In addition to receiving inputs from other clock neurons, the sLNvs also respond to environmental inputs. For example, entrainment to light/dark cycles is dependent on the blue-light photoreceptor CRYPTOCHROME (CRY) and visual photoreceptors [12]. These input pathways can delay or advance circadian behavior phase, which is predominantly controlled by the sLNvs [13]. The eyes of course require neural communication to reset the sLNvs, but even CRY-dependent photoresponses, which were long thought to be cell-autonomous [14], rely on circadian neuron circuits [15,16]. How the sLNvs respond to all these neural inputs is unknown. CREB (cAMP Responsive Element Binding proteins) family members, identified in the Mizrak study [5], are promising candidates for such function. These proteins are under both circadian and electrical activity regulation, and there is an enrichment of cAMP-responsive elements in the promoters of genes under electrical activity control. Intriguingly, circadian period is lengthened under constant conditions if CREB family members are overexpressed. This can be interpreted as a constitutive delaying signal caused by CREB overexpression. In mammals, CREB is well known to respond to increased electrical activity, including in the suprachiasmatic nucleus (the neural pacemaker in mammals) where it gets phosphorylated and induces *mPer1* transcription in response to light inputs [17–19]. In *Drosophila*, transient increases in electrical activity in response to neural input would lead to increased CREB levels and hence activity, and this would contribute to reset the circadian pacemaker in sLNvs. However, it appears unlikely that *per* is a direct CREB target in *Drosophila*, since *per* mRNA levels seem only weakly responsive to hyperexcitation [5]. Importantly, CREB induction would also help to immediately reprogram the circadian transcriptome, without the need to wait for the circadian pacemaker to adjust to a new phase. Such rapid transcriptome plasticity could prove important for flies to respond efficiently to changes in their environment.

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Affective Neuroscience: Food 'Wanting' Hotspot in Dorsal Striatum

New research has uncovered a micro-domain within dorsal neostriatum where enkephalin surges are triggered by the opportunity to consume tasty foods and where μ-opioid microinjections generate intense motivational 'wanting' to eat without enhancing food 'liking'.

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The increased prevalence of obesity poses a global challenge to health. Over-consumption of aggressively marketed, abundant, cheap, tasty, energy-dense foods is one contributor to intake in excess of metabolic demand [1], resulting in increased attention to neural mediators of food reward. A new study [2], reported in this issue of *Current Biology*, has uncovered a novel brain substrate in dorsal neostriatum that mediates excessive consumption of energy-dense foods, where μ-opioid signalling generates intense motivational 'wanting' to eat without elevating the hedonic impact ('liking') of feeding.

It is well established that signalling via brain μ-opioid receptors has potent effects on feeding and that the ventral striatum (nucleus accumbens) is a critical substrate where opioids exert their rewarding effects [3]. A striking aspect of μ-opioid-induced eating is its specificity for foods rich in sugar, fat, or both [3]. Kelley *et al.* [3] proposed that, whilst normal feeding can occur without release of enkephalin (an endogenous μ-opioid receptor ligand), enkephalin release, by enhancing the pleasure of eating, serves to stimulate intake of energy-dense foods beyond that required to maintain energy balance. In an evolutionary context, a μ-opioid-driven urge to overeat when presented with a calorific food source would serve to increase fat stores, aiding survival in the event of future famine [3]. But in the current food-rich environment, such a mechanism seems more of a hindrance than a help.

A detailed account of the role of accumbens opioid transmission in food reward is emerging, based on the work of Peciña *et al.* [4,5]. The hedonic impact of tastes can be measured objectively by orofacial taste reactivity patterns ('liking' reactions), which are homologous across rodent and primate species [4] and which fluctuate in similar ways to human subjective pleasure during hunger/fullness states. For example, sweet tastes elicit a positive hedonic pattern of reactions including tongue protrusions (licking of the lips) [4].

By selectively stimulating μ-opioid receptors in discrete rat brain regions via local microinjection of μ-opioid agonists and studying the extent to which such manipulations enhance 'liking' reactions, Berridge *et al.* [4,5] have previously identified discrete hedonic 'hotspots': micro-domains where μ-opioid receptor stimulation powerfully increases 'liking' reactions to sweet tastes. One such hotspot (1 mm^3 volume) resides in the medial-dorsal accumbens shell [4,5]. Another (0.8 mm^3 volume) resides in a caudal zone of the ventral pallidum, chief accumbens output target [4,5]. These hedonic hotspots act in concert to increase 'liking' reactions to sweet sensations [4,5]. The hedonic hotspots are also 'wanting' hotspots, in that the same microinjections of μ-opioid agonist simultaneously increase both 'liking' and motivational 'wanting' for food, as reflected in vigorous eating [4,5]. The tight localization of opioid hedonic hotspots contrasts strikingly with a looser distribution of substrates, encompassing almost the entire medial accumbens shell (plus amygdaloid regions), where μ-opioid stimulation generates only 'wanting' to eat (large increases in food intake) without enhancing 'liking' reactions (pure 'wanting' hotspots) [4,5]. Treatment with μ-opioid agonists/antagonists also elevates/suppresses consumption of sweet and fatty foods in the wider ventral striatum [3–5].

The new study [2] reveals that μ-opioids stimulate food 'wanting' without enhancing food 'liking' not only in ventral, but also in the dorsal striatum, a region seldom associated with reward. The dorsal striatum has a mosaic organization comprising island-like striosomes/patches embedded in a more extensive matrix [6]. Striosomes are distinguished from matrix by a dense concentration of μ-opioid receptors [6]. Orbitofrontal, cingulate and insular cortices preferentially innervate striosomes, which form part of a 'limbic' circuit embedded in sensorimotor and associative striatum [6]. DiFeliceantonio *et al.* [2] focused on the medial dorsal striatum, which, like the accumbens, contains neurons responding to food [7], is enriched in μ-opioid receptors [8], receives amygdaloid projections [9], and receives body-state signals from lateral hypothalamus via midline thalamic projections [3].

In vivo measurements are critical for revealing normal functioning of opioid transmission within striatal networks. Microdialysis is one approach for *in vivo* studies; however, until recently, measurement of opioid-peptide release in behaving animals has been stymied by problems with recovery and detection sensitivity [10]. DiFeliceantonio *et al.* [2] adopted a novel analysis method — capillary liquid chromatography coupled off-line to multistage mass spectrometry, to determine endogenous opioids in microdialysis samples collected *in vivo*. This technique has been validated for measurement of opioid peptides, showing high sensitivity and specificity [10]. Using this